ESTIMATION OF THE NUMBER OF GENES IN THE GERMINATION ABILITY AT LOW TEMPERATURE IN RICE*

— Genetical studies in rice plant, LVII —

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Introduction

In the rice plant a set of linkage groups which corresponds to the haploid number of chromosomes has now become available, and a diagramatic illustration of the map was also presented by the writers (TAKAHASHI and KINOSHITA 1968). This report is connected with a beneficial side effect of rice linkage markers. It is needless to say that correlation between marker genes and agronomic characters will provide a positive way for elucidating gene systems and for improving varieties in some important characters that are difficult to identify among the segregation products of hybrids, under ordinary cultivating conditions.

Cool weather damage is not so infrequent in Hokkaido, the northern most island of Japan. The damage becomes severe when cool or low temperature comes over at the germination and the ensuing seedling stage and then at the reproductive growth stage. Testing methods to evaluate the degree of cool tolerance at the above respective stages have been devised by the writers and the others (TORIYAMA and FUTSUHARA 1960, SASAKI *et al*) 1970.

In the previous paper of the writers (SASAKI 1970), it was suggested that the germination ability, shortened as germinability, of rice seed at low temperature is an inherent and one of the so-called quantitative characters. The magnitude of expression of this character varies depending on the

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environmental conditions including the physiological conditions of the seed itself. A threshhold-like phenomenon in respect of the relationship between the degree of germinability and the temperature also blurs the intrinsic nature of this character. These are the situation of this character, and therefore it has been a sort of difficulty to analyse genic systems of this character.

As an aid of understanding the part of genic system, the writers made crosses between markers and varieties with several degree of germinability at low temperature. Then, in the hybrid populations, the mode of correlation between marker genes and the degree of germinability were examined. This paper is focussed on the results obtained in this examination.

Befor going further the writers wish to express their appreciation to Dr. Y. SHIMAZAKI, Director of Kamikawa Prefectural Agricultural Experiment Station for his valuable suggestions and encouragement throughout the studies.

TABLE 1. List of the linkage testers and marker genes used

Tester	Marker genes[1] (linkage group)
A- 5	C^{Br}(I), *Pr*(II), *A*·*Rd*(III), *Rc*(IV), *I-Bf*(V)
A- 26	C^{Bp}(I), d_2(II), *Pr*$^+$(II), A^+(III)
A- 83	C^{Bp}(I), *Pr*(II)
A-107	C^{Bp}(I), *Pr*(II), *A*·*Rd*(III), *Rc*(IV)
A-134	C^+(I), A^a(III)
C- 19	*g*(IV), d_1(VI)
H- 9	d_2(II), *bc*(XI)
H- 21	*Rc*(IV), *sh*(VIII), bl_1(X)
H- 45	C^+(I), *wx*(I), *lg*(II), *Pn*·*A*(III)
H- 60	C^B(I), *wx*(I), *Pr*·*lg*(II), A^a(III)
H- 69	C^B(I), A^+(III). *fs*(VII), *nl*(IX)
H- 79	C^B(I), *lg*·d_2(II), A^+(III), *la*(VIII), *bc*(XI)
H-100	C^{Bp}(I), *Pl*(II), *A*(III), d_6(IV), *gl*(XII)
H-126	C^{Bp}(I), *Pl*(II), *A*(III), d_6(IV), *Hg*(XII)
H-143	C^+(I), *gw*·*gh*(VI)
H-145	d_6·d_7(IV), *gw*(VI), *bc*(XI)
H-230	C^+(I), *A*(III), *fs*·*Ur*(VII), *nl*(IX)
H-242	C^+(I), *A*(III), d_6(IV), *fs*(VII), *Dn*(VII)
H-309	C^+·*wx*(I), *A*(III), d_6(IV), *fs*(VII), *Hg*(XII)
N- 4	C^B(I), *Pl*(II), A^a(III), *g*(IV)
N- 45	C^{Bp}(I), *Pl*(II), *A*(III), d_6(IV)
N- 58	d_8(XI)
N- 60	d_9(I)
N- 62	C^+(I), A^a(III), d_{12}(*)

* Linkage group are not decided yet.

1) As to the character expression of these genes, refer to TAKAHASHI and KINOSHITA's paper (1968).

TABLE 2. List of cross combinations used.

Year	Cross	
	No.	Combination
1970	1	H-100 × A- 83
	2	H-143 × A-107
	3	H-143 × A- 5
	4	N- 45 × N- 62
	5	N- 58 × N- 62
	6	H-143 × N- 62
	7	H- 45 × N- 66
	8	H- 45 × H-126
	9	C- 19 × A- 26
	10	H- 21 × N- 62
	11	H- 69 × N- 62
	12	H- 60 × A-134
1971	13	N- 58 × Hayayuki
	14	C- 19 × Hokuto
	15	Chikanari × A-107
	16	H- 60 × Shirayuki
	17	N- 45 × Iburiwase
	18	H-145 × Sakigake
	19	H- 5 × Shisetsu
	20	H-143 × Nohrin 33
	21	Datechikanari × H- 79
	22	H- 45 × Wasebozu
	23	N- 60 × Hashiribozu
1972	24	A- 5 × N- 4
	25	A- 5 × H-143
	26	H- 69 × H- 79
	27	H-100 × A- 5
	28	H-100 × H- 69
	29	A- 5 × H-230
	30	H-242 × A- 5
	31	H-309 × A- 5

Materials and Methods

The marker genes and the cross combinations used in this examination are listed in Tables 1 and 2. As shown in these Tables F_2 populations of the 31 crosses were employed. F_1 plants of these crosses were grown in the ordinary paddy field outdoors, and the F_2 seeds borne in the F_1 plants

were stored in the cool chamber at 5°C for about six months.

Prior to the test of germination ability at low temperature, F_2 seeds were soaked in the water at 2°C in order to remove the so-called water absorption phase. In this examination the "germinability at low temperature" were represented by the "germination date" and "germination coefficient" at 15°C for 12 days. These are based on the reason mentioned in the previous paper of the one of the writers (SASAKI 1970). In facts the most of the seeds germinated from 8 to 10 days after seeding. About 500 seeds were used for each cross in 1970, and from 200 to 250 seeds in 1971 and 1972. Germinated seeds were assorted with their degree of germination dates and were transplated in the nursery bed in the "vinyl house".

Among characters dealt with in the present experiments, those by the genes *Pl* (Purple leaf), *gw* (green and white stripes) and *fs* (fine stripes in leaf margin) made their appearence in the seedling stage, while in the other characters their expression became visible at the maturing period. The experiments were conducted at the field of Kamikawa Prefectural Agricultural Experiment Station from 1970 to 1972. Table 3 is the evaluation of the germinability at low temperature in parental varieties used in the crosses.

TABLE 3. Parental varieties and their germinability at low temperature

Germinability at low temperature	Germination coefficient at 15°C	Name of variety
High	28~38	Chikanari, Sakigake, Iburiwase, Hokuto
Fairly high	24~28	H-45, H-60, H-100, N-62, Hayayuki, H-126, Nohrin-33
Middle	20~24	H-143, A-5, N-4, A-83, A-134, H-230, H-242, H-309
Fairly low	18~20	H-79, H-9, A-26, H-69, H-21, Shinsetsu
Low	8~18	N-66, A-107, Wasebozu, Shirayuki, Hashiribozu, Datechikanari, C-19, N-58, N-45

Experimental Results

The data summarized on the correlation between the markers and the germinability are diagramatically presented in Table 4. In this table, the symbols "++" and "+" indicate the significant differences between two genotypic groups, viz. the group involving the dominant allele and the group with homozygous recessive allele of the concerning markers, at 1% and 5% levels respectively. The symbol "—" means the non-significant difference between the two groups. In a case where significant difference is in existence, it may be natural to concider that a linkage relation between the

TABLE 4. Genetic associations between markers and germinability at low temperature

Year	Cross No.	Linkage group I			II				III			IV				V	VI			VII			VIII		IX	X	XI		XII		*
		Genes *C*	*wx*	d_9	*Pl*	*lg*	*Pr*	d_2	*Pn*	*A*	*Rd*	*Rc*	*g*	d_6	d_7	*I-Bf*	*gw*	*gh*	d_1	*fs*	*Dn*	*Ur*	*la*	*sh*	*nl*	bl_1	*bc*	d_8	*gl*	*Hg*	d_{12}
1970	1	–			–					–				–																	
	2																–	+													
	3																+	–													
	4	–												‡																	–
	5																											–			–
	6																–														‡
	7	–	‡			–			‡																						
	8	–	–		–	–								+																–	
	9							‡											–												
	10											–												–		–					–
	11	+								+										–					–						+
	12		+			–	+																								
1971	13																											‡			
	14												–						–												
	15	–					–				–	+																			
	16					–																									
	17	–			–									–																	
	18													+	‡												–				
	19							‡																			–				
	20																–	‡													
	21	–				+		‡															–				‡				
	22	–	+			–			–																						
	23			‡																											
1972	24				‡		–				–	–	–			+															
	25	+									–	–				–	–	–													
	26					–		–												–			–		–		–				
	27				–		–				–			+		–													–		
	28				–									‡						–					–				–		
	29	–									–	–				–				–		–			–						
	30	–	–		–		–				–	–		‡		–				–	–										
	31	–	+		‡		–				+	–		–		+				–							–			–	

+, ‡ significant difference between dominant and recessive types, at 5% and 1% level, respectively.
– non-significant difference. * linkage group are not decided yet.

marker gene and a gene or genes which have something to do with the germinability at low temperature brings about this result, or the pleiotropic effect of the said marker gene participates.

As shown in the table, there were no remarked association between the germinabiliy and those genes which assigned in the linkage group of VII, VIII, IX and XII. They are *Ur* (Undulated rachis), *fs*, *la* (lazy), *sh* (shattering), *nl* (neck leaf), *gl* (glabrous) and *Hg* (Hairly glumes).

In the linkage group III, which includes three markers, *Pn* (Purple node), *Rd* (Red pericarp) and *A* (Activator for anthocyanin production), significant differences were recognized in three cross combinations out of ten. In the linkage group VI, in which the genes *gw*, *gh* (gold hull) and d_1 ("daikoku" dwarf) belong, there were significant differences in three cases out of eleven crosses. The significant differences were observed in two cases out of seven crosses in the linkage XI which includes the marker

TABLE 5. Linkage relations between the germinability at low temperature and the markers *wx* and *I-Bf*

Cross	Marker	Germination date at 15°C											Total	Mean	Sig.
		2	3	4	5	6	7	8	9	10	11	12			
H- 45×H-126	*wx*	5	10	2									17	2.82	−
	+	13	32	3									48	2.79	
H- 60×A-134	*wx*	10	10	4	3	1	0	0	0				28	3.11	+
	+	18	28	16	11	6	2	1	6				88	3.99	
H- 45×N- 66	*wx*	19	49	30	13	4	2	0	0				117	3.49	++
	+	79	205	56	8	1	2	0	1				352	3.03	
H- 45× Wasebozu	*wx*		1	7	18	12	5	3	2	1	1		50	5.82	+
	+		0	16	46	30	15	15	12	2	11		147	6.45	
H-242×A- 5	*wx*			3	3	3	6	7	4	1	0	1	28	7.18	−
	+			10	9	13	16	8	8	8	3	4	79	7.24	
H-309×A- 5	*wx*			12	18	11	4	5	2	1	0	0	53	5.66	+
	+			38	47	35	19	17	11	8	7	5	187	6.32	
A- 5×N- 4	*I-Bf*			15	23	45	25	19	11	4	4	2	148	6.61	+
	+			2	5	9	23	10	2	3	2	1	57	7.19	
A- 5×H-143	*I-Bf*			4	20	18	32	6	3	1	0	1	85	6.41	−
	+			7	29	35	14	15	8	6	4	3	121	6.72	
H-100×A- 5	*I-Bf*			5	8	14	7	4	3	2	1	1	45	6.56	−
	+			33	32	25	35	31	12	11	8	4	191	6.75	
A- 5×H-230	*I-Bf*			4	5	6	9	4	2	3	2	1	36	7.06	−
	+			21	39	39	30	27	13	7	7	1	184	6.60	
H-309×A- 5	*I-Bf*			5	6	5	0	0	1	0	0	0	17	5.24	+
	+			45	59	41	23	22	12	9	7	5	223	6.25	
H-242×A- 5	*I-Bf*			0	0	0	2	2	1	0	0	0	5	7.80	−
	+			13	12	16	20	13	11	9	3	5	102	7.20	

Notes are the same with those of table 4.

genes, *bc* (brittle culm) and d_8 ("nohrin-28" dwaf). With respect to the gene bl_1 (brown discoloration of leaves and glumes) of the linkage group X, no difference was shown. However this was resulted from only one cross, therefore the further study is required to ascertain the propriety of this relationship.

In summarysing these results, it may be pointed out that there were genetic associations between the germinability at low temperature and such marker genes as *wx* (waxy endosperm), d_2 ("ebisu" dwarf), d_6 ("lop-leaved" dwarf) and *I-Bf* (Inhibitor for brown furrows in glumes), which belong to the following four linkage groups; I, II, IV and V. Some parts on the actual data on the effect of the marker genes in relation to the germinability at low temperature are shown in the Tables 5 and 6. From these tables, there is a tendency that the recessive phase of the gene loci, the *wx* and the *I-Bf*, showed higher germinability than their alternative phases, the

TABLE 6. Genetic relations between the germinability at low temperature and the dwarf genes, d_2 and d_6

Cross	Marker	Germination date at 15°C											Total	Mean	Sig.
		2	3	4	5	6	7	8	9	10	11	12			
C- 19×A- 26	d_2		0	12	22	19	6	2					61	5.38	++
	+		8	75	66	30	6	5					190	4.82	
H- 9× Shinsetsu	d_2			0	0	0	2	5	3	1	0	12	23	10.22	++
	+			2	2	7	8	16	14	16	5	5	75	8.6	
Datechikanari ×H- 79	d_2			0	1	4	7	5	5	8	10		40	8.83	++
	+			1	11	23	54	14	13	11	12		139	7.53	
H- 69×H- 79	d_2			0	6	11	7	9	4	2	2	1	42	7.31	−
	+			2	36	42	19	30	13	6	5	2	155	6.90	
N- 45×N- 62	d_6	32	48	32	4	1							117	3.09	++
	+	202	143	17	3	1							366	2.52	
H- 45×H-126	d_6	4	10	5									19	3.05	+
	+	14	34	0									48	2.71	
N- 45× Iburiwase	d_6		8	11	9	3	1	0	0	1			33	4.48	−
	+		28	41	16	7	1	0	0	0			93	4.05	
H-145× Sakigake	d_6			2	5	22	14	0	1	2	2		48	6.54	++
	+			19	80	29	9	1	1	0	2		141	5.33	
H-100×H- 69	d_6			4	5	7	12	10	8	5	4	2	57	7.67	++
	+			23	33	39	19	22	15	6	6	2	165	6.58	
H-100×A- 5	d_6			9	9	6	9	9	5	7	3	2	59	7.19	+
	+			29	31	33	33	26	10	6	6	3	177	6.55	
H-242×A- 5	d_6			1	1	5	2	5	4	5	0	3	26	8.27	++
	+			12	11	11	20	10	8	4	3	2	81	6.89	
H-309×A- 5	d_6			8	12	10	9	5	4	2	2	0	52	6.40	−
	+			42	53	36	14	17	9	7	5	5	188	6.11	

Notes are the same with those of table 4.

phases including dominant alleles (Table 5), while the reverse relations were found in the d_2 and d_6 loci (Table 6). In other words, Table 5 shows that the germination days of the recessive type are significantly lower than that of the dominant one, and Table 6 shows that the germination days of the recessive type were significantly higher than that of the dominant one.

In the dwarf genes, it is noted that the significant differences were found in most of the crosses examined. The difference of the germination days between normal and the dwarf types were more prominent comparing with those between the dominant and the recessive alleles in the *wx* and the *I-Bf* loci.

Discussion

TAKAHASHI (1962) studied the two kinds of genetic relations. They are between the genes for germination at low temperature (20°C) and the genes for the permeability of iodine uptake, and between the genes for germinability and a gene for pericarp color. He concluded that these genes are genetically independent with the gene for germinability. LEE (1970) also studied the relations among genes, those which are responsible for the germinability at low temperature, the apiculus color and the awning. There were no remarkable linkage relation between the germinability at low temperature and apiculus color, while a weak linkage relation was indicated between the genes for awning and the germinability. In the present examination, genetic associations were found out between the genes for the germinability at low temperature and the marker genes, *wx* (the linkage group, I), d_2 (II), d_6 (IV) and *I-Bf* (V). Therefore, the genes for the germinability at low temperature are assigned to four of the twelve linkage groups, suggesting that at least four or more gene pairs are concerning to the germinability at low temperature.

As to the intrinsic nature of the correlation between the germinability and the dwarfness, there remaines a possibility of pleiotropic effect of the dwarf genes, since the correlation between the germinability at low temperature and the elongation of the initial seedling growth which was also found by one of the writers (SASAKI 1968). He, further, pointed out some relations between the germinability at low temperature and growth regulator substance.

The linkage relations between the quantitative charaters and the marker genes were examined by TORIYAMA and FUTSUHARA (1960), FUTSUHARA and TORIYAMA (1966). They reported the results that the genes for cool tolerance at the reproductive stage associated with the genes, d_2, *bc*, *nl* and

gh. This line of experiment was followed by one of the present writers (TAKAHASHI 1968). In his examinations at least eleven markers of seven linkage groups showed association with cool tolerance, suggesting that the genic system of this character would be complex.

In connection with the inference of the number of genes controlling the germinability at low temperature the following datum should be added. One of the writers, SASAKI, tried to estimate the number of effective factors for this character through the biometrical analysis by MATHER's method,

TABLE 7. Scaling test for mean days to germination at 15°C

Generation	Mean days to germination	±	S.E.
P_1	3.7988	±	1.3833
P_2	8.8455	±	2.0889
F_2	5.5263	±	2.6776
F_3	6.1889	±	1.7340
MP	6.3222	±	1.5597
A	1.0587	±	6.4751

$A=4\bar{F}_3-\bar{P}_1-\bar{P}_2-2\bar{F}_2 \qquad V_A=16V_{\bar{F}_3}+V_{P_1}+V_{P_2}+4V_{\bar{F}_2}$

TABLE 8. Estimates of variance components for the germinability at low temperature

Item	Variance component	Observed	Expected	Deviations
V_{F_2}	$\frac{1}{2}D+\frac{1}{4}H+E_1$	5.2846	4.9933	0.2931
$V_{\bar{F}_3}$	$\frac{1}{2}D+\frac{1}{16}H+E_2$	1.8628	1.8625	0.0003
$\bar{V}_{F_3}$	$\frac{1}{4}D+\frac{1}{8}H+E_3$	2.9199	3.5066	−0.5867
Non-heritable components	E_1	3.1687	3.4621	−0.2634
	E_2	0.4639	0.4632	0.0007
	E_3	3.3290	2.7402	0.5868

Estimate of each components			±	
	D	2.7122	±	2.5349
	H	0.6904	±	4.6937
	E_1	3.4621	±	0.6223
	E_2	0.4632	±	0.6560
	E_3	2.7422	±	0.5081

$$E_1=\frac{V_{P_1}+V_{P_2}}{2},\quad E_2=\frac{V_{\bar{P}_1}+V_{\bar{P}_2}}{2},\quad E_3=\frac{\bar{V}_{P_1}+\bar{V}_{P_2}}{2}.$$

using the data of F_2 populations and P_1, P_2, F_3 lines of the cross "Iburiwase ×Hokkai 95". The results obtained are given in Tables 7 and 8.

As a result of the scaling test, it has been shown that the mean days to germination is regarded as an adequate in dealing with the present character of which concerning genes are acting additively with the similar effects (Table 7). The component of the variation D, H, E_1, E_2 and E_3 were estimated by a least square technique, using V_{F_2}, V_{F_3}, $\overline{V}_{F_3}$ and E_1, E_2, E_3 (Table 8).

Here, the following genic assumptions were made; i) one of the parents (Iburiwase) posses all of the dominant alleles and the other (Hokkai 95) has all of the rscessive ones, ii) the high germinability is dominant to low germinability $\left(\frac{h}{d}=0.5045\right)$, and iii) there were no linkage relations between these genes. In these bases, the number of effective factors, K_1 and K_2, were estimated as;

$$K_1=\frac{(\bar{P}_1-\bar{P}_2)^2}{4D}=2.3477$$

$$K_2=\frac{{}_H\overline{V}^2_{F_3}}{{}_H\overline{V}_{VF_3}}=0.9385$$

As mentioned above, and in the case where the distribution of allelomorphs is isodirectional in the parental lines, K_1 is not reduced in value from lack of full concentration. Thus we have;

$$K=K_1(1+V_\alpha)=K_2(1+V_\beta)$$

then putting $r=\frac{V_\alpha}{V_\beta}$

$$V_\alpha=\frac{K_1-K_2}{rK_2-K_1} \quad \text{and} \quad K=\frac{K_1K_2(r-1)}{rK_2-K_1}$$

when V_α is small and $r=4$

$$V_\alpha=\frac{K_1-K_2}{4K_2-K_1} \quad \text{and} \quad K=\frac{3K_1K_2}{4K_2-K_1}=4.7002$$

The number of factors estimated by MATHER's procedure were fairly well coincided with the results obtained in the present experiment of the linkage relationships. However, there were some cases in which the results obtained vary from cross to cross, and the different varieties and strains posess different genes. These results suggest that the genetic mechanism controlling the germinability would be complex, and therefore further study should be accumulated in this respect.

Summary

The present study carried out to obtain further information of genetic mechanism on the germinability at low temperature and the linkage analysis by the use of linkage testers.

Thirty-one, crosses between the linkage testers and the varieties or strains which indicated different degree of germinability at low temperature were used for the present experiment. The germinability at low temperature were expressed as a germination date at 15°C.

As the results, genetic correlations were found out between the germinability at low temperature and such marker genes as *wx*, d_2, d_6 and *I-Bf*, in the linkage groups of I, II, IV and V, respectively. Therefore, the number of gene loci connected with the germinability was estimated to be four or more. This number of genes were coincided with the number of effective factors responsible for this character, estimated by MATHER's biometrical procedure, through a particular cross combination in which two parental varieties showed two extremes of the degree of germinability at low temperature. Data obtained in the present examination suggest that the genetic mechanism controlling this character would be complex.

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